CARBAPENEM RESISTANCE MECHANISMS IN NON-CARBAPENEMASE-PRODUCING *Pseudomonas aeruginosa*

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Introduction

Pseudomonas aeruginosa is the most frequent microorganism in nosocomial infections affecting mainly immunocompromised patients[1] . High mortality rates are associated with the ability of this pathogen to develop antimicrobial resistance.
Carbapenems are the first choice of treatment for *P. aeruginosa* MDR infections, as they are stable to most beta-lactamases, including extended spectrum beta-lactamases[2]. Carbapenem resistance is often associated with enzymatic hydrolysis by carbapenemases[3]. However, in the absence of carbapenemases, the loss of OprD is the most prevalent mechanism among carbapenem resistant *P. aeruginosa*, followed by overexpression of efflux pumps and/or chromosomal AmpC[4]. This study aims to investigate the mechanisms of carbapenem resistance in six isolates of non-carbapenemase-producing *P. aeruginosa*, which belong to two different sequence types (STs) according to previous study[5].

Method

Six carbapenem-resistant *P. aeruginosa* (CRPA) non-carbapenemase-producing and five carbapenem-susceptible (CSPA) strains were analyzed in this study. All carbapenem-resistant strains, as well as one susceptible strain, were typified previously by MLST in two different STs, ST2236 and ST2237. These isolates are part of a collection of 35 strains, from recovered burned patients and balneotherapy tanks in a previous work. MICs for imipenem and meropenem were determined in all strains by using M.I.C.Evaluator™ strips (epsilometric test) according to manufacturer's instructions. *P. aeruginosa* ATCC27853 was used as control. For carbapenem-resistant strains, MICs for both antibiotics were also determined by broth microdilution method, according to the Clinical and Laboratory Standards Institute (2018)[6], and phenotypic detection of efflux and AmpC overexpression was assessed by MIC reduction in the presence of the efflux pump inhibitor (PAβN) or in the presence of the AmpC inhibitor (cloxacillin). Mutations in *oprD* sequence were investigated in six CRPA strains and one CSPA by whole amplification of these genes through PCR, followed by sequencing. Sequencing analyses were performed using Lasergene Software and compared with the PAO1 reference strain. To analyze the expression of *mexA*, *mexX*, *mexC*, *mexE*, *oprD* and *ampC* genes, transcriptional levels of these genes were obtained through RT-qPCR for all CRPA (with and without induction by imipenem) and CSPA strains. *P. aeruginosa* PAO1 was used as reference strain.

Results / Discussion

CRPA strains showed MIC values ranging from 16 to >32 µg/mL for imipenem and ≥32 µg/mL for meropenem by using epsilometric test[7], except for the carbapenem

resistant strain “24”, which was susceptible to meropenem and presented MIC= 2 µg/mL for this antibiotic. MIC values were also determined by broth microdilution method for all CRPA strains. These strains showed MIC=16 µg/mL for both carbapenems. Only the meropenem susceptible strain “24” showed MIC=1 µg/mL for this antibiotic. These results displayed moderate levels of resistance for both meropenem and imipenem in CRPA strains. The phenotypic test using the inhibitors PAβN and cloxacillin did not detect overexpression of efflux pumps and AmpC, respectively for both carbapenems. Except for the strain 31, which showed a significant reduction in meropenem MIC in the presence of PAβN. Sequencing of *oprD* gene revealed both indel and point mutations in the analyzed strains with similar mutation patterns in strains of the same ST. No indel mutation was detected in the *oprD* gene of ST2236 strains (3, 24 and 26), only base pair substitutions. However, a premature stop codon was detected only in the resistant strains of this ST (3 and 24). Mutations in *oprD* gene were particularly impactful in ST2237 strains (2, 4, 5 and 31), since indel mutations were found causing loss of porin. Failure to quantify *oprD* transcripts by RT-qPCR further confirms the absence of functional porin on ST2237 strains. ST2236 strains showed low transcriptional levels for *oprD* including the susceptible strain (26). No overexpression was detected for the efflux genes, although the *mexX* gene showed increased expression in 83% of resistant strains. High transcriptional levels (> 10X) of *ampC* were found in 50% of non-induced CRPA. All induced CRPA strains showed an increase in *ampC* expression, in the order of 10^2-10^3 times higher than non-induced strains. This result highlighted the importance of AmpC overexpression in carbapenems resistance in the evaluated strains, since *oprD* reduced expression was present in both CRPA and CSPA strains. Loss of OprD only confers imipenem resistance in *P. aeruginosa* if AmpC β-lactamase is expressed[8].

Conclusion

The reduction and/or loss of OprD porin associated with AmpC overexpression seemed to likely be the main determinants of resistance to carbapenems in the evaluated strains. Although efflux pumps overexpression has not been observed, *mexX* increased expression may have contributed to this resistance phenotype.

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